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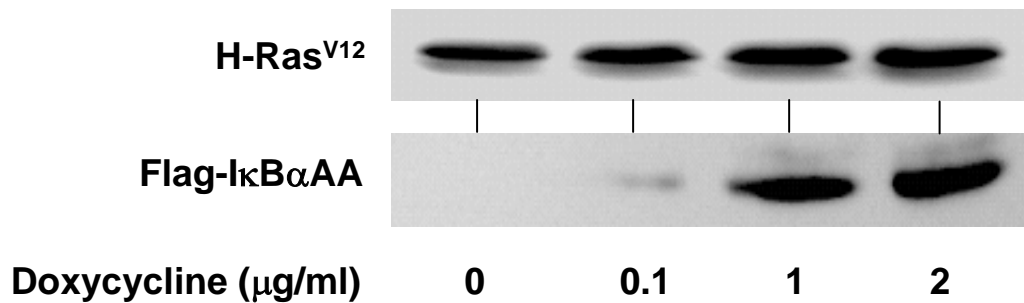
A

LTR5'—Neo^r—TB-NF κ B-Luci—rtTA—LTR3'

Doxycycline ↓

LTR5'—Hyg^r—CMV-- H-ras^{V12}—TetO—Flag-I κ B α AA—LTR3'

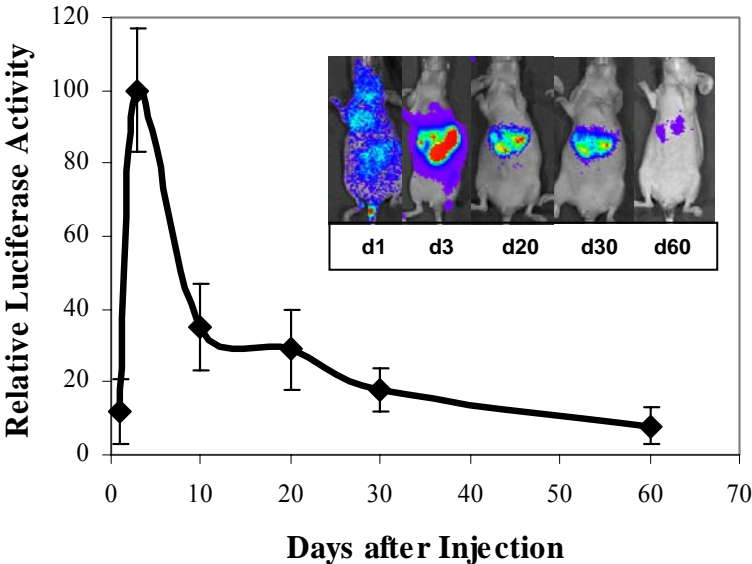
B



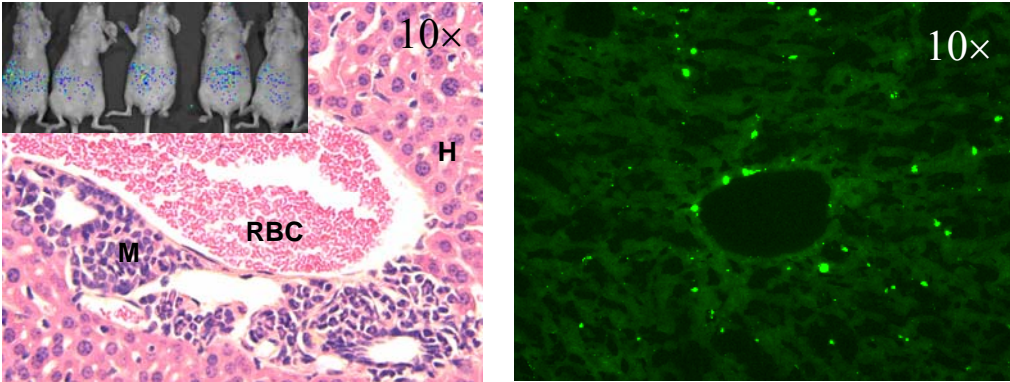
Supplementary Figure 1 Legend. A, Scheme for the Ta-NF κ B luciferase retroviral vector (upper panel) contains the NF κ B promoter sequence linked to a luciferase reporter gene and the tetracycline transactivator (rtTA). The Tr-Ras-I κ B retroviral vector (lower panel) contains a CMV promoter which allows the constitutive expression of H-ras^{V12} followed by the tight Tet ON responsive promoter which regulates the expression of the Flag tagged mutant I κ B α AA. The inducible expression of I κ B α AA will occur when the tetracycline transactivator (rtTA) binds to the TetO promoter in the presence of doxycycline. B, Cell lines stably expressing retroviral inducible vectors. The primary INK4A/ARF^{-/-} mouse melanocytes were sequentially infected with above packaged retroviral vectors. A single colony was selected as described in Materials and Methods. Western blot analysis was performed for cells constitutively expressing H-Ras^{V12} protein and inducibly expressing Flag-I κ B α AA protein in the presence of doxycycline.

Supplementary Figure 2

A



B



Supplementary Figure 2 Legend. A, Liver is the main target of the EBV-based p4486 expression vector. The luciferase gene was cloned into the p4486 vector linked to CMV promoter to facilitate evaluation of the duration of expression of the EBV-based vector *in vivo*, 100 µg DNA of the p4486-luciferase vector was injected i.v. into nude mice. These mice were given luciferin i.v. at the indicated time points and subsequent luminescent imaging was performed (right insert) and quantitated (right graph). B, The liver is the major site of melanoma lesions after iv injection of tumor cells. Nude mice were injected i.v. with 2×10^5 Ras-melanoma cells. Sixty days after injection, the luminescent images (inserted in the left panel) and histological analysis (H&E staining of left panel) indicated liver was the main site for melanoma lesions. H, hepatocytes; M, melanoma cells; RBC, red blood cells. Melanoma cells stably expressing GFP were injected i.v. into nude mice. Thirty days after injection, frozen sections were examined under the fluorescent microscope, directly indicating the liver as the major site of melanoma growth (right panel). B, dRzIKK reduction of NF-κB activity in the hepatic melanomas. 2×10^5 Ras-melanoma cells were injected i.v. into BALB/C-*nu/nu* female mice (n=10/group). These mice were given dRz vector DNA (100 µg/mouse) i.v. every 30 days. Eighty days after injection of cells, mice were subjected to luminescent imaging